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PATENT
1060-0144P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: Balázs SÜMEGI Conf.: 8470
Appl. No.: 10/084,095 Group: 1614
Filed: February 28, 2002 Examiner: Spivack, P.
For: PHARMACEUTICAL COMPOSITION HAVING
ENHANCED ANTITUMOR ACTIVITY AND/OR
REDUCED SIDE EFFECTS

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Balázs SÜMEGI, residing at 7634 Pécs, Homokkő u. 7.
HUNGARY, do hereby declare as follows:

I am a citizen of HUNGARY.

I graduated from the Jozsef Attila Univ., Szeged, Hungary in
1975 with a M.S. in chemistry. In 1978, I obtained a Ph.D. in
biology (biochemistry) from Univ. Medical. Sch. Pécs, Hungary. In
1990, I obtained a D.Sc. in biology (biochemistry) from the
Hungarian Academy of Sciences, Budapest.

I have been engaged in research activities since 1975. I am
currently a Full Professor and Chairman, University of Pécs,
Faculty of Medicine, Department of Biochemistry. I was also the
Dean of Faculty of Medicine at the University of Pécs from 1996-
1999. I am an author or co-author of 68 peer-reviewed journal
articles. A copy of my Curriculum Vitae is attached.

I have read and understand the subject matter of U.S. Application Serial Number 10/084,095 and have carried out or supervised the following experiments relating to the invention described therein:

The following tests were completed to illustrate the effect of compound L on the side effects of fluorouracil.

Reduction the gastrointestinal toxicity of fluorouracil

Fluorouracil is widely applied in the cytostatic treatment of colorectal, breast, head and neck cancers. However, its dose-limiting gastrointestinal and bone marrow toxicity prevents the use of higher, possibly more effective doses in clinical practice. In the following experiments, the toxic effect of fluorouracil on the small intestine was studied.

Female Balb/c mice of 6-weeks old were used in the tests. The animals obtained standard laboratory chow and tap water ad libitum. Fluorouracil was diluted with physiological saline and applied ip. alone or in combination with compound "L".

The control group was treated with physiological saline, and compound "L" dissolved in physiological saline was administered to a further group of animals ip. Each test group consisted of 9 mice. The possible clinical symptoms and the body weight of the animals were checked daily.

Forty-eight (48) hours after the treatment, the animals were sacrificed, the small intestines of each were removed, washed

outside and intraluminally with distilled water containing 154 mM of sodium chloride and 1 mM of dithiothreitol (DTT) in 1 liter volume. The weight and length of the intestines were measured and thereafter the intestines were frozen in liquid nitrogen. In addition to the intestinal relative weight, the damage to the small intestine was characterized by the activity of two enzymes: thymidine kinase and sucrase. On the day of enzyme measurements, small intestines were pulverized in liquid nitrogen and the powder was suspended in isotonic aqueous potassium chloride solution containing 0.3 % DTT. The total homogenate was used for the determination of the sucrase activity, and the cytosol obtained after centrifugation ($100,000 \times g$) was used for the determination of thymidine kinase activity.

The activity of thymidine kinase characterizes the decrease of the biosynthesis of the DNA synthesis precursor deoxythymidine monophosphate (dTMP). The sucrase activity characterizes the reduction of the digestive capacity of the mucosa after cytostatic treatment. The determinations were carried out as described in the article: Bagrij, T. et al., "Influence of uridine treatment in mice on the protection of gastrointestinal toxicity caused by 5-fluorouracil", *Anticancer Res.*, 13:789-94 (1993). The obtained thymidine kinase activity values are given as nmole dTMP/hour/cm intestine, while the obtained sucrase activity values are given as μ mole glucose/hour/cm intestine. The latter results together with the intestinal relative weight (in

g/cm intestine) are summarized in the following table.

Treatment groups	Intestinal relative weight in g/cm	Sucrase activity in μ mole glucose/hour/cm	Thymidine kinase activity in nmole dTMP/hour/cm
Control	0.021	6.57	0.83
Compound "L" 200 mg/kg	0.022	6.61	0.85
Fluorouracil 84 mg/kg	0.018	4.26	0.50
Fluorouracil 84 mg/kg + compound "L" 200 mg/kg	0.021	5.52	0.67
Fluorouracil 112 mg/kg	0.017	3.93	0.45
Fluorouracil 112 mg/kg + compound "L" 200 mg/kg	0.020	5.81	0.63
Fluorouracil 150 mg/kg	0.016	3.85	0.40
Fluorouracil 150 mg/kg + compound "L" 200 mg/kg	0.020	6.16	0.58

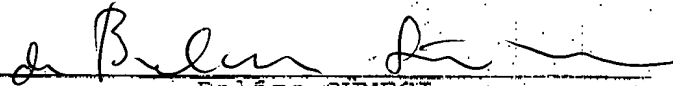
After cytostatic treatment, the intestinal relative weight represents the general damage and the progressive destruction of the small intestine epithelium. It can be seen from the above table that increasing doses of fluorouracil from 84 to 150 mg/kg caused a dependent decrease of the intestinal relative weight in monotherapy with fluorouracil. The administration of compound "L" alone gave a similar result as the control group. However, the combined therapy with increasing doses of fluorouracil and 200 mg/kg of compound "L" essentially restored the intestinal relative weight to that of the control group.

A similar dose dependant decrease in sucrose and thymidine kinase activity was observed during monotherapy with fluorouracil. Although the simultaneous addition of compound "L" could not restore the original activity values, a significant amelioration can be seen in the dose range studied. Thus, it can be concluded that a combined therapy with fluorouracil and compound "L" can effectively reduce the gastrointestinal toxicity of fluorouracil even when fluorouracil is administered in a dose of as high as 150 mg/kg.

I hereby declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated this 4 day of June, 2004.

Signed



Balázs SÜMEGI

CURRICULUM VITAE

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Education

M. S. Chemistry - 1975 -Jozsef Attila Univ. Szeged, Hungary
Ph.D. Biology (Biochemistry) - 1978 - Univ. Medical. Sch.
Pecs, Hungary.
D.Sc. Biology (Biochemistry) 1990 Hungarian Academy of Sciences,
Budapest.

Professional Appointments

1975-1978 Biochemist with Dr.I.Alkonyi, Univ. Med. School,
Inst. of Biochem. Pecs, Hungary

1978-1980 Postdoctoral Fellow with Dr.I.Alkonyi,Univ.Med. School,
Inst. of Biochem. Pecs, Hungary

1980-1992 Assitant Professor at Univ.Med.Sch.Inst.of Biochem. Pecs, Hungary

1983 Jun-1984 Jun Visiting Scientist, Univ.Texas Health Science Center,
Dept.Biochem. Dallas, TX, USA

1985 Oct-1986 Oct Visiting Scientist at Univ.Texas Health Science Center,
Dept. of Biochem. Dallas, TX, USA

1986 Oct-1987 Oct Research Chemist,Veterans Administration, Medical Center,
Pre-Clinical Science Unit. Dallas, TX, USA

1989-1991 Visiting Professor, Univ.Texas at Dallas,
Department of Chemistry, Richardson, USA.

1992-1994 Associate Professor, University Medical School Pecs,
Department of Biochemistry, Pecs, Hungary

1994-present Full Professor and Chairman, University of Pecs,
Faculty of Medicine Department of Biochemistry.

1996-1999 University of Pecs, Dean of Faculty of Medicine

Professional Societies

Hungarian Society of Biological Chemistry. Member of Executive Committee.

Teaching Experience

Lecturing medical students from biochemistry and Biomedical application of NMR.

Leader of Biochemistry and Molecular Biology Ph.D program.

Symposia and Meeting(as invited participant)

1/19-23-87 Gordon Conference-Santa Barbara,CA, USA

4/17-24-89 UCLA Symposia. Keystone, Colorado, USA

5/1-5-89 Scanning Microscopy. Salt Lake City, USA

10/16-20-90 Third International Meeting on the Function of
Thiamine Diphosphate Enzymes, Blaubeuren,Germany

1/20-26-91 Gordon Conference-Oxnard, CA, USA

9/6-18-92 FEBS Advanced Course, Application of NMR Techniques
to probe Metabolism in Yeast and other Organisms.

14-09-98 Gordon Research Conference, Oxford

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2. Alkonyi,I., Gyocsi,L., and **Sumegi,B.**: Demonstration of a Lag Period in the Timecourse of the Reaction Catalyzed by Pyruvate Dehydrogenase Complex. **Acta. Biochim. Biophys. Acad. Sci. Hung.** 13, 253-258, 1978.
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5. **Sumegi,B.** and Alkonyi,I.: Paracatalytic Inactivation of Pig Heart Pyruvate Dehydrogenase Complex. **Arch. Biochem. Biophys.** 223, 417-424, 1983.
6. **Sumegi,B.** and Alkonyi,I.: Elementary Steps in the Reaction of Pyruvate Dehydrogenase Complex from Pig Heart. **Eur. J. Biochem.** 136, 347-353, 1983.
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10. **Sumegi,B.** and Srere,P.A.: Complex I Binds Several Mitochondrial NAD-coupled Dehydrogenases. **J. Biol. Chem.** 259, 15040-15045, 1984.
11. **Sumegi,B.**, Batke,J. and Porpaczy,Z.: Substrate-induced Structural Change of the Pyruvate Dehydrogenase Multienzyme Complex. **Arch. Biochem. Biophys.** 236, 741-752, 1984
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15. Srere,P.A. and **Sumegi,B.**: Organization of the Mitochondrial Matrix. In: Miocardial and Skeletal Muscle Bio-energetics,(Brautbar,N., ed), Vol. 194, pp 13-25, **Plenum Press.** 1986.

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